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Donald F. Adams, et al

Washington State University
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DEVELOPMENT OF A FLUORIDE-SPECIFIC MICROELECTRODE

Donald F. Adams and Jerry D. Price

Washington State University

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Introduction

Macroscopic aspects of visible damage to plants during fluoride accumulation have been quite well described. Specific sites of fluoride accumulation within plant tissue are less well known. Attempts to follow the dynamic progression of fluoride through plant tissue have been unsuccessful because of a lack of discriminative fluoride detection techniques. Although much research has been undertaken by many researchers during the past decade to elucidate the mechanism of fluoride action within plant tissue, such methods as microautoradiography[1] have been unsuccessful in following the dynamic movement of fluoride through fluoride-treated foliar tissue.

The general areas of accumulation of fluoride within plant tissue have been grossly defined. By 1950, chemical analyses of incremental portions of exposed foliar tissue showed that the fluoride concentrates in the tips and margins of the leaves[2]. Later, studies with radioactive fluorine-18[1] confirmed this gross storage pattern and indicated a preferential storage of fluoride in the cell walls in chloroplasts and soluble proteins.

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The penetration of radioactive F^{18} through plant epidermis was also studied[1]. Because of the relatively short half-life (112 min) and the relatively long exposure required to produce an autoradiogram (18 hours), the dynamic movement of fluorine within the plant tissue cannot be plotted. These investigators made several unsuccessful attempts to locate the sites of fluorine accumulation within the various possible components of leaf cells by microradiography. Failure of this technique was reported to be "probably due to the high water solubility of the fluorides"[1].

At best these experimental techniques can only provide "after-the-fact" storage site data and cannot reveal the dynamic progress and movement of the fluoride within the plant structure.

Thus it is obvious that previously used techniques are inadequate for study of the dynamic movement of fluoride through plant tissue, through cell walls and into or out of plant cells.

A new technique is required to reveal this dynamic movement of fluoride through plant cellular structure. A fluoride-specific, micro-electrode, if available, could be selectively inserted into plant cells and provide the means for monitoring the dynamic movement of fluorides at the cellular level.

Therefore, to obtain more exact information on the site(s) of fluoride accumulation, as the free anion or in a Van der Waals association, we proposed to apply both the theory and technology of microelectrodes to the development of a fluoride ion-specific microelectrode for the study of the movement of fluoride ions through the plant cellular structure.

Experimental evidence is accumulating that plants exposed to fluorides at concentrations below which visible leaf injury is produced may have an altered carbohydrate mechanism[42,43]. McCune, et. al. states that "glucose

dissimulation shifts from the Embden-Meyerhof - Parnas pathway to the pentose phosphate pathway because of one or more of the following reasons: a. increasing maturity of the tissue[44,45], b. the effect of fluoride per se [37,45,46,47], c. in the absence of additional fluoride influx, the amount of fluoride present at the metabolic sites decreases[48] and/or d. the fluoride present in the tissue is converted to an inactive form[43]."

Confirmation of the validity of one or more of these reasons for the observed shift in metabolic pathway would be made possible by monitoring the dynamic movement of fluoride through the plant cellular structure with fluoride ion-selective microelectrodes.

Other experimental evidence indicates that reduced levels of calcium[44] and nitrogen [45] in the tissue result in increased sensitivity of plants to fluoride-induced injury. Fluoride-specific microelectrodes could be an invaluable tool in providing a quantitative explanation for these observed phenomenon.

The availability of a micro probe which could detect and record the movement of the fluoride ion through various plant tissues would provide another link in the chain of knowledge which McCune, et. al.[43] believe to be necessary to interpret adequately "the significance of a change in one metabolic step for the metabolism and physiology of the plant as a whole and the relation of metabolic changes to injury or reduced yield" as these changes are related to fluoride influx into plant tissue.

Much new technology has been developed since Ling and Gerard first reported the direct measurement of cell membrane potentials with a glass micropipette electrode 22 years ago[3].

Although extracellular electrodes had been effective in detecting differences in membrane properties occurring between different parts of the

muscle cells, it was not until the development of intercellular microelectrodes with open tips of 1 micron (10^{-4}cm) or less diameter that probing within cells without disrupting the natural functions of the cell was permitted[4,5]. These developments have resulted in the definition of the role of sodium and potassium exchange in the electrical activity of muscle[6,7].

Membrane electrode technology for ion specific electrodes for a wide range of ions has seen a rapid development since the discovery of the H^+ sensitive glass electrode by Cremer[8] and Haber and Klemensiewicz[9] at the beginning of the century. Five decades later, Eisenman and his colleagues[10-13] reported the development of specialized glasses specifically selective to ions such as Na^+, K^+, Ag^+, NH_4^+, Tl^+, Li^+, and Cs^+. We have seen the recent development and use of liquid-liquid ion exchange membranes for divalent cations[14] and a variety of anions[15]. Macroelectrodes based on solid-state crystals have been developed[16], and a new variety of specific-ion membranes is just emerging, based upon the use of organic macromolecular complexes using typically cyclic polyethers[17], antibiotics[18-21], enzymes[22] or bicyclic amines[23], which offer the possibility of exquisite selectivities of biological membranes[18,24,25]. The simple heterogeneous membrane coated wire electrode has recently been shown to be responsive to ion activity changes for Cu^{++}[26] and Ca^{++}[27].

The activity of iodide ions has been selectively measured with a membrane macroelectrode prepared by embedding silver iodide in a paraffin or silicone rubber matrix[28]. With this electrode, iodide ion activity has been measured even in the presence of "million-fold excesses" of chloride. The membrane potential measured is proportional to the logarithmic value of the quotient of ion activities on both sides of the membrane[29] as predicted by the Nernstian equation as applied to ion-specific electrode responses.

Orion Research Inc. has developed a fluoride-specific solid state macroelectrode[30] which it has marketed for approximately four years. Although the precise production method of the crystalline membrane is not known, the single crystal consists of lanthanium (III) fluoride doped with 5% europium (II) fluoride[31]. This electrode is obviously not directly applicable to intercellular use because of its overall size. The early measurements on semi-micro samples (50 μl) were accomplished by simply inverting the Orion electrode[32], however this method could not be used for measurements in situ but, rather the sample had to be placed in the microelectrode cell. Later[33], a semi-microelectrode for fluoride was constructed from the Orion type with a 300 micron tip o.d. This allowed application to in situ measurements, however, there is no fluoride-specific, microelectrode system (tip o.d. of less than 5 microns) presently available for in vivo measurements.

Several investigators have prepared cation- and anion-specific microelectrodes by filling the tips of micropipettes with a liquid organic ion exchanger. These electrodes yield near Nernstian response down to approximately 10^{-5} molar as shown by the calcium selective electrode, for example[34].

The heterogeneous membrane fluoride ion-specific electrodes[35] that were forerunners to the solid-state model would appear to have a membrane structure which could be more simply reduced to the required micro size than the single crystal membrane.

We propose to investigate and construct miniaturized chemical systems which will specifically respond to fluoride within individual plant cells or elsewhere.

In the approach to the development of fluoride ion-specific microelectrode for use in plant tissues, two types of problems can be expected to occur: 1. those relating to the nature of the solution to be tested and 2. those relating to the electrode. Problems that can arise in testing a biological system in vivo for fluoride activity will put specific stresses on the production of the microelectrode.

What are the conditions within plant tissue which cause us to believe that fluoride-specific microelectrodes, could be used to measure the dynamic movement of fluoride through tissue? Ion-selective membrane electrodes have been shown to be of use in fast-flow studies of biological reactions[36], i.e. their response time is rapid. A somewhat uniform solute concentration exists throughout the cytoplasm and extracellular solution[4]. Thus the passage of fluoride through this "uniform" solute should change the electrochemical characteristics of this solute and be measurable, if suitable microdetectors were available. The detector electrode must have a higher specificity ratio for fluoride than other anions that are likely to be present, i.e. chloride, nitrate, sulfate, and phosphate, Then, by plotting the progress of fluoride from the atmosphere through the extracellular solution to the cytoplasm, its freedom of movement and its possible fixation of inactivation within the cell walls for vacuoles as nonionic calcium or aluminum fluoride[37] could be determined. There is also evidence that some inorganic ions tend to accumulate in the vacuole in concentrations in excess of 100 times the surrounding solutions[38]. There is little information concerning the relative concentration of fluoride within the different cellular structures and the various plant fluids. Experiments with etiolated pea stems have shown that 3×10^{-3} M NaF did

not affect respiration[39] whereas 5×10^{-3} M NaF inhibited respiration changes. If only one hundredth of this concentration were present in the cell, it could be easily measureable with a fluoride ion-specific electrode.

The problems that arise in the electrode production actually fall into different orders of seriousness depending upon the type of membrane used. However, no matter which type of membrane is used, a fairly high specificity for fluoride must be of utmost importance. There have been two general types of membranes which can be considered: 1. solid and 2. liquid-liquid ion exchange.

Solid membrane, ion-specific electrodes have been widely studied and the mechanism by which they function well developed[40]. Macroelectrodes with solid fluoride ion-selective membranes are available commercially[30] and the miniaturization of these require only the production of a single crystal of the proper size. It is presumed to be a valid assumption from the reduction of this type of electrode to a semi-micro scale[33] that its functioning characteristics would not be seriously altered in reduction. However, the reduction of the cross-sectional interface does result in a rapid increase in the resistance of electrode. This same objection must be considered valid for heterogeneous, solid, fluoride ion-selective membranes; although it can be noted that it is easier to produce the miniaturized heterogeneous membranes.

Liquid-liquid ion exchange membranes seem to be a logical choice to solve such a problem. As stated by Eisenman[40], the resistivity of such a membrane will be lower than its solid state counterpart, as the ion exchange sites are now mobile and can move between the two

interfaces yielding a greater conductivity. Because this electrode works on a solvent extraction type of principle, specificity becomes the major problem.

If the specificity of a biological membrane can be approached in the membrane, then the electrode would be of invaluable use. Therefore, biological compounds such as enzymes[22] and antibiotics [18-21] might be great value. Liver esterase, for example, is inhibited by fluorides in the nanogram concentration range. On the negative side, antibiotic membranes are known only for cations (usually K^+) and many enzymes are also sensitive to other anions particularly phosphate. Fungal laccase does appear to be an extremely promising possibility, as it contains a cupric ion (type 2, "non-blue", paramagnetic) which has a very strong affinity for fluoride ions[41]. The use of an encapsulating compound, such as a macrobicyclic amine[23], in a liquid membrane to produce a high selectivity for the small diameter fluoride ion also appears to be a reasonable approach.

The use of the coated wire type electrode [26,27] is limited by the strength of available wires. It is not believed at this time that a wire and membrane with a tip o.d. of 5 microns or less could be produced that would be durable enough to puncture the plant tissue.

Experimental

A proposal to study methods for production of a fluoride-selective microelectrode and the subsequent use of such an electrode to follow the movement of fluoride ions within plant tissue was first prepared for submission in 1963. This proposal was rejected as being impractical and too broad in concept. Between 1963 and the funding of this present study

in 1969, much progress has been made in other laboratories in the production of ion-selective macroelectrodes. The practicality of ion-selective electrode processes has been firly established.

Our initial laboratory study began with the preparation of membrane precipitate type macroelectrodes, as pioneered by Pungor[29] and Mac Donald[35], designed to have I^-, Cl^-, F^-, or PO_4^{-3} selectivity. This approach permitted us to develop our electrode production techniques and to compare the response of these electrodes with published data and theory.

Precipitates of AgI, AgCl, Ag_3PO_4, or LaF_3 were suspended in silicone rubber (G.E. Clear Seal). Films were formed over an open end of 10 mm I.D. glass tubes. The electrodes were usually filled with 10^{-3}M solutions of the sodium or potassium salt of the anion of the precipitate studied. Silver wire provided the internal electrical contact. The reference electrode was generally AgCl/Ag. All of these macroelectrodes, with the exception of the LaF_3 electrode, gave reproducible Nernstian response over a concentration range of 10^{-5} to 10^{-2}M (see Figure I).

To determine the possible influence of size scale-down upon the response of similar membrane-type macroelectrodes, we successfully prepared chloride and phosphate solid-state microelectrodes in glass capillaries, as above.

Glass microelectrode blanks were prepared by pulling 0.8 mm Pyrex tubing with a standard electrode puller. The tip diameters of these microelectrode blanks ranged from 1 - 2 microns. The electrode blanks were first filled with methanol by immersing them tip up in heated absolute methanol for approximately 5 minutes. The heated methanol and microelectrodes were then placed in a dessicator for 5 minutes under partial vacuum. The electrode blanks were stored under methanol until ready to

be filled with the appropriate electrolyte and precipitate. The methanol was replaced by the appropriate electrolyte by repeated (3 times) heating and cooling under vacuum with the methanol filled electrode blank standing tip up in the electrolyte solution.

The microelectrode tips were filled with the precipitate by centrifugation. A machined Teflon electrode support provided support for six electrode blanks at a time. The support fit into a 1-inch diameter X 50 ml centrifuge tube. The lower portion of the centrifuge tube was filled with appropriate electrolyte, the Teflon holder and capillaries were placed in the tube and a suspension of the precipitate was placed on top. The tube was then centrifuged for an hour at $\sim$2500 rpm. The precipitate produced a plug at the tip of the capillary blanks. Continuity of each plug was determined by microscopic examination. The acceptable electrodes were stored under electrolyte until used.

The chloride- and phosphate-selective microelectrodes responded to variations in Cl^- and PO_4^{-3} ions respectively according to theory (Figure II). Both electrode types were evaluated for response to other ions. The chloride microelectrode did not respond significantly to di- and trivalent ions over the concentration range (Figures III-V) of 10^{-4} to 10^{-1}M concentrations of the interfering ions. Similarly the phosphate microelectrode did not respond to mono- and di-valent ions over the same concentration range. The chloride microelectrode shows response to bromide ion (Figure VI) above concentrations of 10^{-4}M, equivalent to that produced by chloride. The electrode also shows a response to pH changes (Figure VII), although the response cannot be correlated to the Nernstian equation and must be considered to be a result of the relative hydration of the electrode

tip as it varies with pH and its effect on the mobility of the chloride ion or other subtle effects.

The successful production of chloride- and phosphate-selective microelectrodes showed that precipitate type ion-selective electrodes can be scaled down to a 1 - 2 micron tip diameter without impairing the anticipated ion-selective response.

Hinke[46] has reported the successful preparation of H^+, Na^+, K^+, and Cl^- microelectrodes. This chloride microelectrode has a "working diameter" of 5 - 10 μ and is of the Ag/AgCl type. Figure VIII compares the measured response of the Hinke chloride ion-selective microelectrode a. with the 2μ chloride ion-selective electrode produced in this laboratory, b. and the Orion chloride ion-selective macroelectrode, c. which is commercially available. Although, the response of the two microelectrodes (a and b) are similar and shows the same magnitude as the Orion model (c), it will be noted that the response is in the opposite direction; i.e. a ten-fold decrease in the concentration produces an approximate increase of 55 millivolts in the Orion model, but a decrease by the same amount in the potential produced by either of the microelectrodes over their working range. It is apparent, however, that the slopes of all three of the potential curves show near Nernstian response and the change in sign may be a result of a change in the internal and reference electrode system.

During the time which the microelectrodes were being examined, a study into what may have caused the poor results in the case of the lanthanium fluoride electrode was begun. Study of the LaF_3 precipitation process indicated that the laboratory preparation of LaF_3 in aqueous media probably yielded hydrated or oxyfluoro-species with a widely varying composition.

A literature search revealed that LaF_3 may be prepared by any one of four methods 1. the reaction of the oxide with anhydrous HF, 2. the reaction of the oxide with NH_4HF_2, 3. the precipitation of the fluoride from aqueous solution, or 4. the passage of anhydrous HF through the molten fluoride salt[47].

Because of the specialized conditions and equipment required to produce satisfactory anhydrous lanthanium (III) fluoride, the possibility of obtaining it from a commercial source was investigated. Mac Donald[35] reported, however, that membrane electrodes which he prepared from commercially available pure lanthanium fluoride were almost entirely unresponsive to changes in fluoride ion concentration. He theorized that commercially produced anhydrous rare earth fluorides are calcined and thus may be deactivated for electrochemical use.

Possible methods of producing suitable anhydrous lanthanium (III) fluoride will be investigated further. In addition, other possible fluoride ion-selective membranes have been and are presently still under investigation. These include both inorganic fluoride salts and organic ion exchange type of membranes.

There are three major criteria which must be evaluated as a guide to choosing materials which can be used as membranes in ion-selective electrodes. These are, of course, a. solubility, b. selectivity, and c. conductivity. The proper balance between the three must be found to produce a functional ion-selective electrode. The solubility, for the case of our work, must be significantly less than 1 ppm, the lower concentration range likely to be found in plants. The membrane compounds used must have a low enough solubility that equilibrium can be established without

changing the concentration of fluoride in the plant tissue being tested. It was probably for this reason, at least partially, that Orion[30] chose to use lanthanium (III) fluoride as the membrane in their commercially available fluoride ion-specific macroelectrode.

Lanthanium(III) fluoride has a reported K_{sp} of $\sim 10^{-29}$ moles/liter, however, solubility product data is derived from data obtained for an equilibrium between a saturated solution and the freshly prepared powder form of the solid. It can be logically reasoned[40] that, a single crystal, with its lower surface area, is likely to have an even lower solubility than that reported in solubility data and therefore makes a better membrane for an ion-selective electrode. Other salts to be investigated must have a solubility of less than at least $\sim 10^{-14}$ moles/liter from K_{sp} data to be of useful consideration for membrane materials.

The second criterion is selectivity. Ideally the membrane will selectively allow the measurement of only the ion of interest. The limitation of the membrane to a small number of ions to which it will respond is of extreme importance. If it were to respond to several, even if not to the same magnitude of the ion of interest, the interference from changes in secondary ions could give a completely misleading representation of what is occurring in the sample.

In a liquid membrane the selectivity is determined by properties of both the solvent and the ion-site pair, through the organophilic exchanger. However, which terms are significant in producing the membrane depend upon the conditions which are assumed to be present at the membrane surface. The equation for the potential produced across a liquid membrane is[40]:

$$\frac{zF}{RT} E = -\ln \frac{\Sigma u_i K_i a_i''}{\Sigma u_i K_i a_i'} - \int_1 - \int_2$$

where $\int_1$ and $\int_2$ are defined as integrals of specific functions between the two membrane interfaces and under the limiting conditions. The terms have the following difinitions:

u_i - mobility of the i^{th} ion in the solvent phases.

K_i - partition coefficient of the i^{th} ion between water and the solvent.

a' - activity of the i^{th} ion in the test solution.

a" - activity of the i^{th} ion in the internal reference solution.

z - charge of the ion of interest.

F, R, T, and E are the Faraday constant, gas constant, temperature, and potential respectively.

Now if we apply the conditions of 1. Steady State - $J_s^{TOT} = 0$; therefore the first two terms determine the membrane potential as the $\int_2$ is zero $\left(\frac{u_s J_s^{TOT}}{\frac{RT}{F}}\, dx\right)$ and the expression simplifies to

$$\frac{zF}{RT} E = -\ln\left[\frac{\int u_i a_i'' K_i}{\int u_i a_i' K_i}\right] - \int_1$$

and 2. Complete dissociation ($IS \rightarrow I^- + S^+$); therefore $C_{is} = 0$ for all 'i' which cause t = 0, since $t = \left[\frac{u_s C_s}{\left(\frac{u_s C_s}{\Sigma u_{is} C_{is}}\right)+1\ \Sigma u_k C_i + u_s C_s}\right]$ and the term $\int_1$ which is $t\int_o^d d\ln\left[\frac{\Sigma u_{is} K_{is} C_i}{\Sigma u_i C_i}\right]$ drops from the expression and the potential becomes $E = \frac{RT}{zF} \ln\left[\frac{\Sigma u_i a_i' K_i}{\Sigma u_i a_i'' K_i}\right]$ or for an ion-selective electrode in terms of the ion of interest:

$$E = \frac{RT}{zF} \ln \frac{a_1' + \Sigma \frac{u_i K_i}{u_1 K_1} a_i'}{a_1'' + \Sigma \frac{u_i K_i}{u_1 K_1} a_i''}$$

The term $\frac{u_i K_i}{u_1 K_1}$ is the weighting factor for the ith ionic species, better known as the selectivity constant. This term can be easily seen to be ratio of the relative products of the ionic mobilities and the partition coeffients for the interferring ion and the ion of interest; fluoride ion for our work. The selectivity is therefore determined entirely by the solvent and is independent of the exchanger, which would be expected from the condition of complete dissociation that was imposed during the derivation of the expression for the potential.

If, however, we consider the case of an encapsulating compound, such as the bicycloamines; strong association is found between the sites and ion species and the above equation is not valid. The equilibrium in the equation:

$$I^- + S \rightleftarrows IS^-$$

lies very far to the right. Therefore, ΣC_{is} is much greater than C_s and $\frac{u_s C_s}{\Sigma u_{is} C_{is}} <<< 1$ and the parameter t simplifies to t_s (the transference number), $t_s = \frac{u_s C_s}{\Sigma u_i C_i + u_s C_s}$ and then $\int_1$ can be written: $\int_o^d t d\ln \frac{\Sigma u_{is} K_{is} C_i}{\Sigma u_i C_i}$ which after some manipulation can be written as $(1-T)\ln\frac{\Sigma(u_i + u_s)a_i'' K_i}{\Sigma(u_i + u_s)a_i' K_i} + T\ln\frac{\Sigma u_{is} K_{is} a_i'' K_i}{\Sigma u_{is} K_{is} a_i' K_i} - \ln\frac{\Sigma u_i a_i'' K_i}{\Sigma u_i a_i' K_i}$ yielding an equation for the potentials of

$$\frac{zF}{RT}E = (1 - T)\ln\frac{\Sigma(u_i + u_s)a_i''K_i}{\Sigma(u_i + u_s)a_i'K_i} + T\ln\left(\frac{\Sigma u_{is}K_{is}a_i''K_i}{\Sigma u_{is}K_{is}a_i'K_i}\right).$$

Note that the above equation requires only the condition that $J_s^{TOT} = 0$ and does not require the counter ions to be in a steady state. The electrode potential of a liquid ion exchanger membrane in which strong association occurs is seen to consist of two logarithmic terms, each of the form of the previous electrode equations. The relative contribution of the two terms is controlled by the value of the parameter, $T(0 \leq T \leq 1)$. The selectivity in the first term of the equation depends on the product of the partition coefficient ratio of the dissociated ion species $\left(\frac{K_i}{K_1}\right)$ and the ratio of the summed mobilities of the dissociated ions and the dissociated sites $\left[\frac{(u_i + u_s)}{(u_1 + u_s)}\right]$. It, therefore, depends predominantly on the solvent and only depends on the nature of the liquid exchanger through its effect on site mobility (u_s). On the other hand, the selectivity in the second term of the equation is determined by the product of the mobility ration of the ion-site pairs $\frac{u_{is}}{u_{1s}}$ and the ion exchange equilibrium constant K_{1i} of the reaction:

$$X_i^- \text{ (aq.)} + X_1S \text{ (mem)} \rightleftharpoons X_1^- \text{ (aq.)} + X_iS \text{ (mem)}$$

which is formally identical to the reaction for the solid state membrane. The equilibrium constant K_{1i} can alternatively be expressed as the product of the partition coefficient ratio of the ions and the association constants in the membrane phase: $K_{1i} = \frac{K_iK_{is}}{K_1K_{1s}}$

The selectivity of K_{1i} depends on the properties not only of the organophilic ion-exchanger molecule but also on the solvent in which it is dissolved.

Eisenman[40] has shown mathematically that the potential for a solid state membrane is described by the equation

$$E = \frac{RT}{zF} \ln \left[a_1 + \frac{\Sigma \frac{u_i}{u_1} K_{1i} a_i'}{\Sigma \frac{u_i}{u_1} K_{1i} a_i''} \right]$$

which gives rise to a rather simple selectivity constant $\frac{u_i}{u_1} K_{1i}$. So, for a solid state membrane the selectivity arises from the nature of the ion as shown in its mobility and by the equilibrium constant K_{1i} for the reaction:

$$X_i^- \text{ (aq.)} + X_1M \text{ (mem)} \rightleftharpoons X_1^- \text{ (aq.)} + X_iM \text{ (mem)}$$

It is expected that the more strongly an ion is preferred by such an ion exchanger, the more poorly it moves once in the membrane; since the more difficult it is for it to jump from site to site. For this reason, there are opposing effects between mobilities and affinities of ions in solid ion exchangers, which constitute one of principle limitations of such systems.

The third criterion for selection of membrane materials is conductivity. This is not a hard feature for even the layman to understand. Conductivity is a measure of the efficiency with which a measurement can be performed. If the conductivity is too low, i.e. a resistivity of more than about 10^{12} ohms, then trying to obtain meaningful data with a normal pH/millivolt meter is at best a questionable operation.

We have already discussed the ease with which conductivity can be maintained across a liquid ion-exchange membrane. The sites carrying the charged ions are free to move between the two interfaces. Now let us look at the solid state membrane.

First there are two types of defects which will allow conduction through a crystal. The Schottky defect is a vacancy or hole within the crystal lattice formed by the deficiency of an ion. The Frenkel defect is the presence of an interstitial ion within the crystal lattice. Both of these defects can be treated as electricity since they can move through the lattice relatively easily. Provided they are controlled by an appropriate equilibrium, both defects may be useful for conduction through electrode membranes.

Therefore, in the consideration of metal fluorides to be used in possible ion-selective membranes the factors to be considered were solubility and conductivity. Early work investigated such compounds as $KLaF_4$, $NaLaF_4$, CaF_2 and BiF_3 and of course Orion[30] had already demonstrated the application of lanthanium (III) fluoride doped with europium (II) fluoride (5%). The lack of a high quality source of lanthanium (III) fluoride caused the $KLaF_4$ and $NaLaF_4$ to be dropped from further study as we could not produce them in a state which was applicable to membrane studies.

Dobbelstein and Diehl[48] seemed to have found a reasonable solution to the salt solubility problem, phenol-formaldehyde polymer membranes into which a metal ion salt of the anion of interest is incorporated.

Bakelite A was produced in a 65°C oven by the addition of equal weights of crystalized phenol and 37% formaldehyde into a covered beaker. The mixture was maintained at 65°C and left covered for 8 hours with stirring

about each half hour. The heating was maintained with stirring every half hour for five more hours after the cover was removed from the beaker. Additional formaldehyde, approximately 20% of the initial amount used, was added to the vessel and the cover replaced. The mixture was now heated, with the cover on, at 65°C for 30 minutes, stirring each 10 minutes. The cover was removed and the mixture heated at 65°C with occasional stirring for about three hours. The heavy viscous liquid was then saved for future use.

The metal salts of fluorides with titanium (IV), bismuth (III), nickel (II), calcium, or aluminum as the metal cation were used to try to produce an ion-selective membrane for fluoride in the following manner. 1 - 2 grams of Bakelite A was dissolved in 20 ml ethanol (acetone used in the case of bismuth (III) fluoride) and an appropriate amount of the fluoride salt added along with ethylene glycol (serving as a plasticizer). The mixture was then placed into the 65°C oven and the excess solvent removed with occasional stirring. When the mixture became a thick slurry it was placed into the tip of melting point capillaries by placing one end of the tube into the slurry and allowing the solution to draw in by capillarity. The capillaries were then placed, membrane down, into a beaker and placed into the 65°C oven and the temperature raised at the rate of 10°C per hour to 130°C. Once the temperature of 130°C was obtained the capillaries were maintained at the temperature for 12 hours, at which time the oven was turned off and allowed to cool to room temperature gradually.

After the capillaries had cooled to room temperature, they were removed from the oven and inspected microscopically to insure efficacy of the membrane. Those capillaries having a usable membrane were place into an

electrolyte solution of 3M KCl, 1M KF, and saturated AgCl with the membrane tip up. The solution was then placed into a vacuum oven at 80°C, a vacuum drawn with an aspirator for one-half hour and then released gradually. This procedure was usually sufficient to fill most of the capillary, however, there was normally a small air bubble against the membrane. This air bubble was removed by inserting a fine platinum wire down the length of the capillary to the membrane and moving the bubble up the wire by tapping gently on the membrane end of the capillary. The filled capillaries were stored under the electrolyte solution until they were ready for testing.

The internal reference was of the silver/silver chloride type, produced by the chloridization of a silver wire in 0.1M HCl solution. The internal reference was then soldered onto a U.S. standard pH electrode plug lead (Figure IX). The reference electrode is a silver/silver chloride electrode (Orion Research Inc., single junction reference electrode, sleeve type, #90-01) and the potential measurements were made with a digital pH meter (Orion Research Inc., Model #701). This is the basic set-up for all electrodes tested as illustrated in Figure X.

The electrodes produced with this type of membrane have been tested in the above situation over a concentration range of fluoride ion from 10^{-1} to 10^{-6}M approximately.

Those membranes using calcium fluoride in salt concentrations of 50, 45, 41, 30, and 20% failed to give a satisfactory response to changes in fluoride ion activity. It must be reasoned that the calcium ion could not form the necessary ligands with the organic polymer to produce an activity ion-selective membrane or as Mac Donald found this calcium fluoride salt was simply not active[35].

The aluminum metal ion was felt to be a more successful choice. At salt concentrations of 50, 30, and 20% by weight, the membrane seemed to lack any reproducibility, however, at 40% by weight aluminum (III) fluoride the response to fluoride ion activity changes were not only reproducible but near Nernstian for relatively concentrated solution (above 10^{-3}M). Unfortunately, the response of this electrode falls rapidly below this concentration and it also shows some response to other univalent anions. Further investigation of this electrode to see whether either or both of these problems might be alleviated is being presently conducted.

The testing of other polymer-inorganic fluoride electrodes is continuing in this laboratory at the present time with both the titanium (IV) and bismuth (III) salts showing some promise.

Hoping to alleviate some of the problems which are inherent in solid state membrane electrodes, we began a search for a possible organophilic ion exchanger to be used in producing a liquid-liquid ion exchange membrane. Of the three criteria, only selectivity is a problem in this type of electrode. If we refer to electrode potential equation derived previously and examine it closely, it is easily seen that the value of T, which must lie between 0 and 1, depending on the properties of the solvent and the exchanger, determines the relative weight of each logrithmic term in the total potential produced.

For a particular system, the potential might be largely determined by either the first or the second term. For example in our case under consideration, if the association constant for the fluoride ion is much larger than that of any other ion, and if the mobilities of the ion-site pairs are approximately equal so the $u_{ls} \cong u_{is}$, then T reduces to $\frac{u_s}{u_i + u_s}$

from which $(1-T)$ is obviously seen to be $\frac{u_i}{u_i + u_s}$ and is seen to be the transference number of the most dissociated counter-ion species and T is identifiable as the transference number of the free site relative to the most dissociated counter-ion species.

If the above conditions are fulfilled and the mobility of the site from one membrane interface to the other is significantly greater than the principle counter-ion, then it can easily be seen that the equation for the membrane potential can be estimated at

$$\frac{zF}{RT} E = T \ln \frac{\Sigma u_{is} K_{is} a'^{K_i}}{\Sigma u_{is} K_{is} a''^{K_i}}$$

From the above equation, it can easily be seen that the potential for interference from secondary ions is directly proportional to their ability to be transferred across the membrane by the site, i.e. a site-ion complex formation. Simmons[23] showed that certain macromolecular, bicyclic amines had the ability to encapsulate halogens with some selectivity. If such a compound could be placed into a liquid-liquid ion exchange membrane that had a selectivity for fluoride, then the conditions for the membrane potential equation estimation are met and the assumptions that follow will be valid.

Simmons[23] noted two compounds during his study that did not form a complex with chloride, bromide, nor iodide ions although they were of the same bicycloamine structure as those he was studying. His data listed one of these compounds with a 'hole' diameter of 2.8 A°, this cavity is large enough to accommodate a fluoride ion (ionic diameter of 2.72 A°) but not

the larger halides or other anions. This compound, 1,10 diazabicyclo - (8,8,8) tetracosane, could then be used to obtain a very high selectively for the fluoride ion, as only its hard ion can be fitted into the diaza i^+, i^+ cavity. The i^+, i^+ conformer is considered to be the most stable

state of the ion-organophilic complex.

We feel that the use of this compound can give us a membrane which will suffer only from hydroxyl ion interference (OH^- ion is also considered to be a hard anion with approximately the same ionic diameter as fluoride) and can, therefore be used in plant tissue, where the pH is nearly constant, without fear of interference from other ions giving misleading results. The synthesis of 1,10 diazabicyclo (8,8,8) tetracosane has begun in this laboratory and with the completion of that step, a working semi-micro model will be constructed to test the validity of these assumptions.

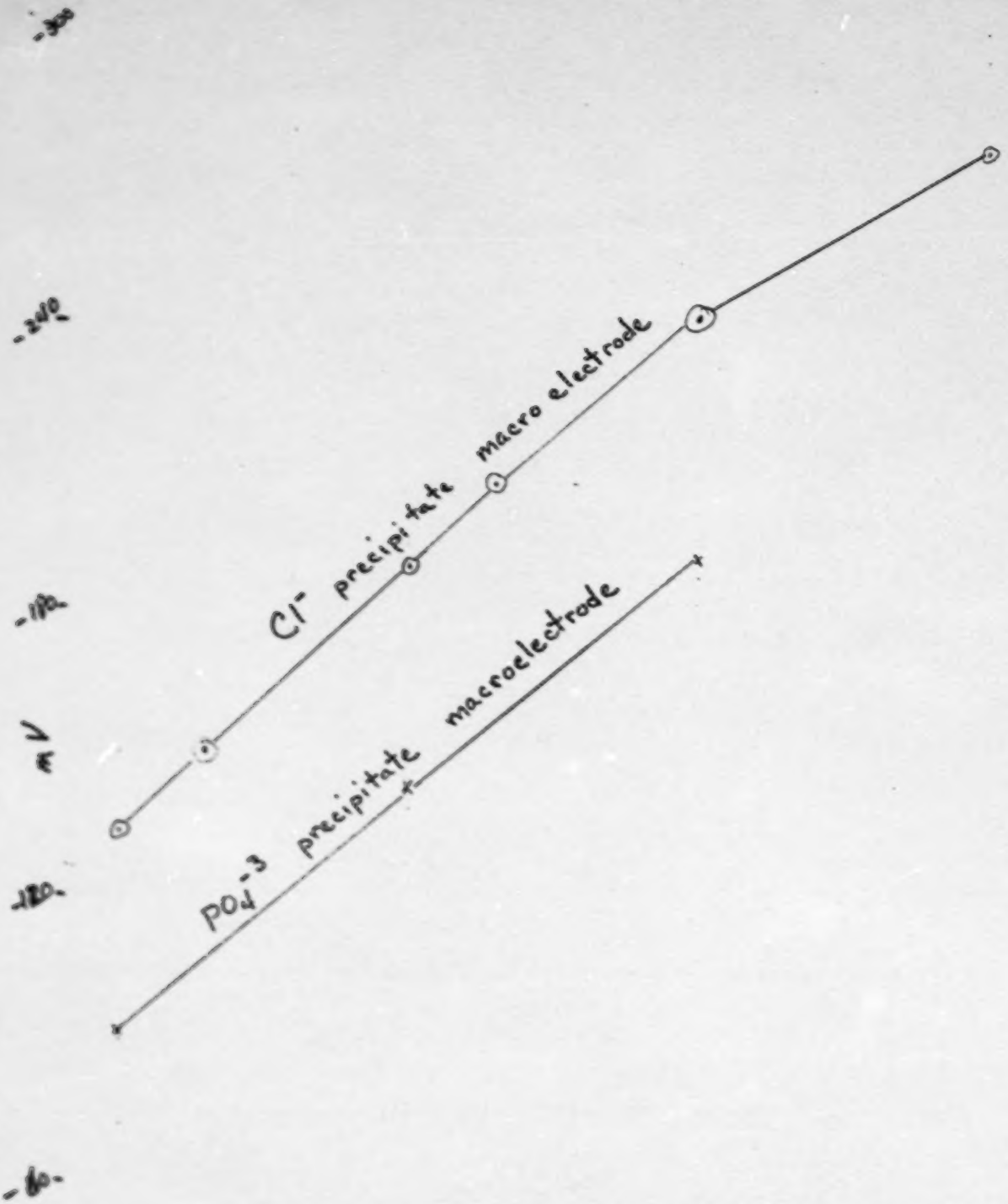
Two other types of electrodes are presently being studied in our laboratory. The platinum wire, coated with an inert resin, PVC in our present study, containing approximately 70% by weight of a fluoride salt represents a simple electrode model. This type of electrode has been shown to work for the divalent cations calcium[27] and copper[26] and functions as the heterogeneous membrane type electrodes do except that it does not contain the added bulk of internal reference system. This advantage does appear to be evident, except that at the required 1 micron tip diameter, the platinum wire will not have the required strength to puncture a plant tissue. However, working models of this type of electrode with a tip

diameter of 500 microns have been produced containing sodium hexafluoroferrate, calcium fluoride, or bismuth (III) fluoride as the fluoride salt. Of these only the bismuth (III) fluoride membrane has shown any real promise as an ion-specific electrode. This membrane did give near Nernstian response of a 10^3 molar change in the fluoride ion activity. On the negative side however, this could not be replicated between the three electrodes produced and some sort of consistancy in the production must be obtained (Figure XI).

Paraffin-salt membranes of calcium fluoride were produced, however, initial tests have shown no response to fluoride at salt concentrations of 50% by weight in the membrane. It is presently felt that Mac Donald[35] probably stated a truth greater than he realized when he reported for calcium fluoride, "As with the other types of membrane electrodes, membranes prepared from precipitates made with excess of anion were entirely inselective, but those with an excess of cation showed some promise." From the work done on inorganic fluoride membranes in this and other laboratories, it would seem evident that in order to function as a membrane compound for an ion-selective electrode the salt must be produced from an excess of the metal ion. If consideration is given to the three properties that a membrane must have, one can easily see why this must be true. The criteria that is affected of course is that of conductivity. By using an excess of metal ions, we can produce a crystal that contains Schottky defects with its lattice, which can and will provide a charge carrying hole for the fluoride ions, i.e. a hole into which a fluoride ion could move.

All the work done in this laboratory has to date used only commercially available inorganic fluoride compounds, which are not made from excess metal

ions but from excess fluoride and therefore do not contain Schottky defects nor suitable electrochemical properties. We are presently undertaking the preparation of calcium fluoride from excess calcium chloride as described by Mac Donald[35] and intend to research the preparation of other metal fluorides from excess metal ions, particularly aluminum and bismuth.



Response of heterogeneous membrane macroelectrodes

FIG. I

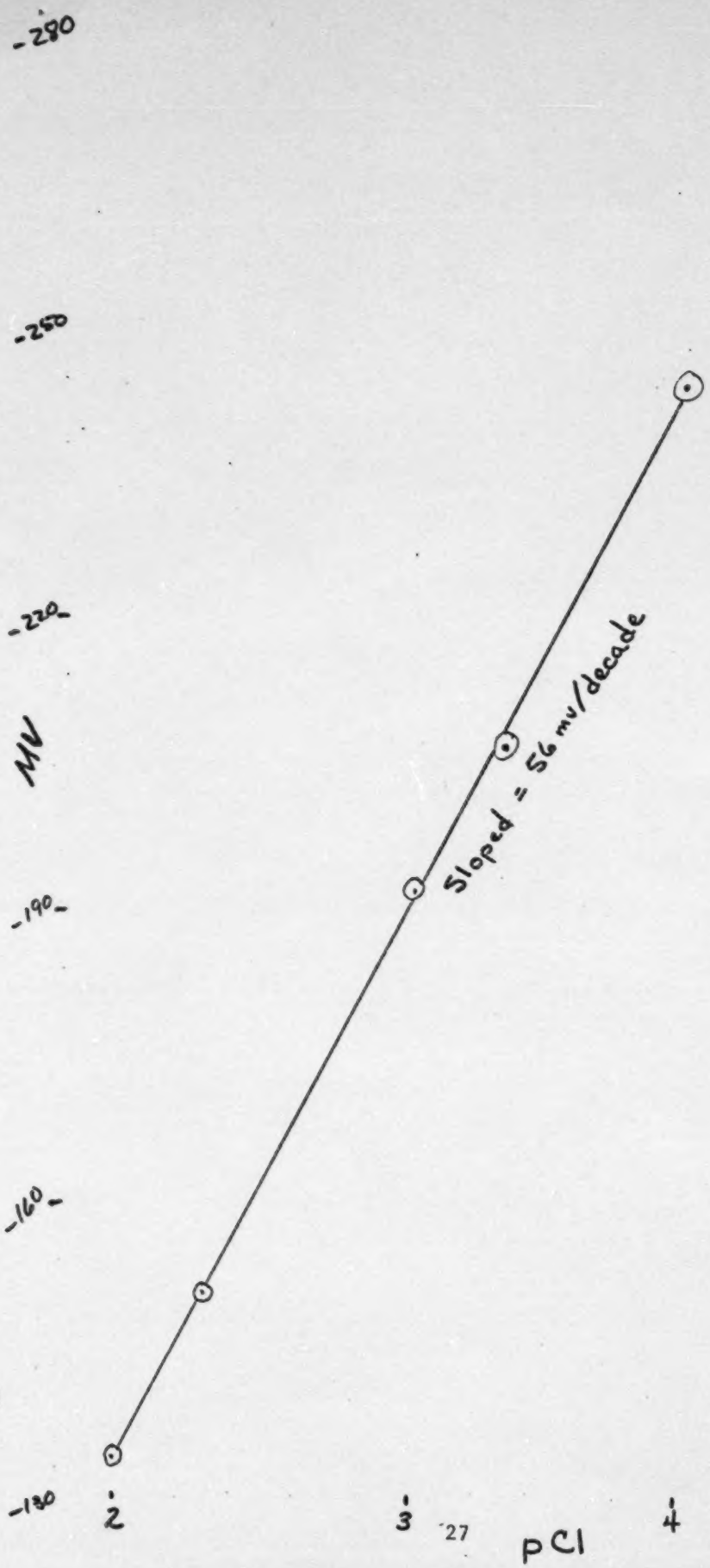


Fig. II

response of Cl^-
2μ tip electrode

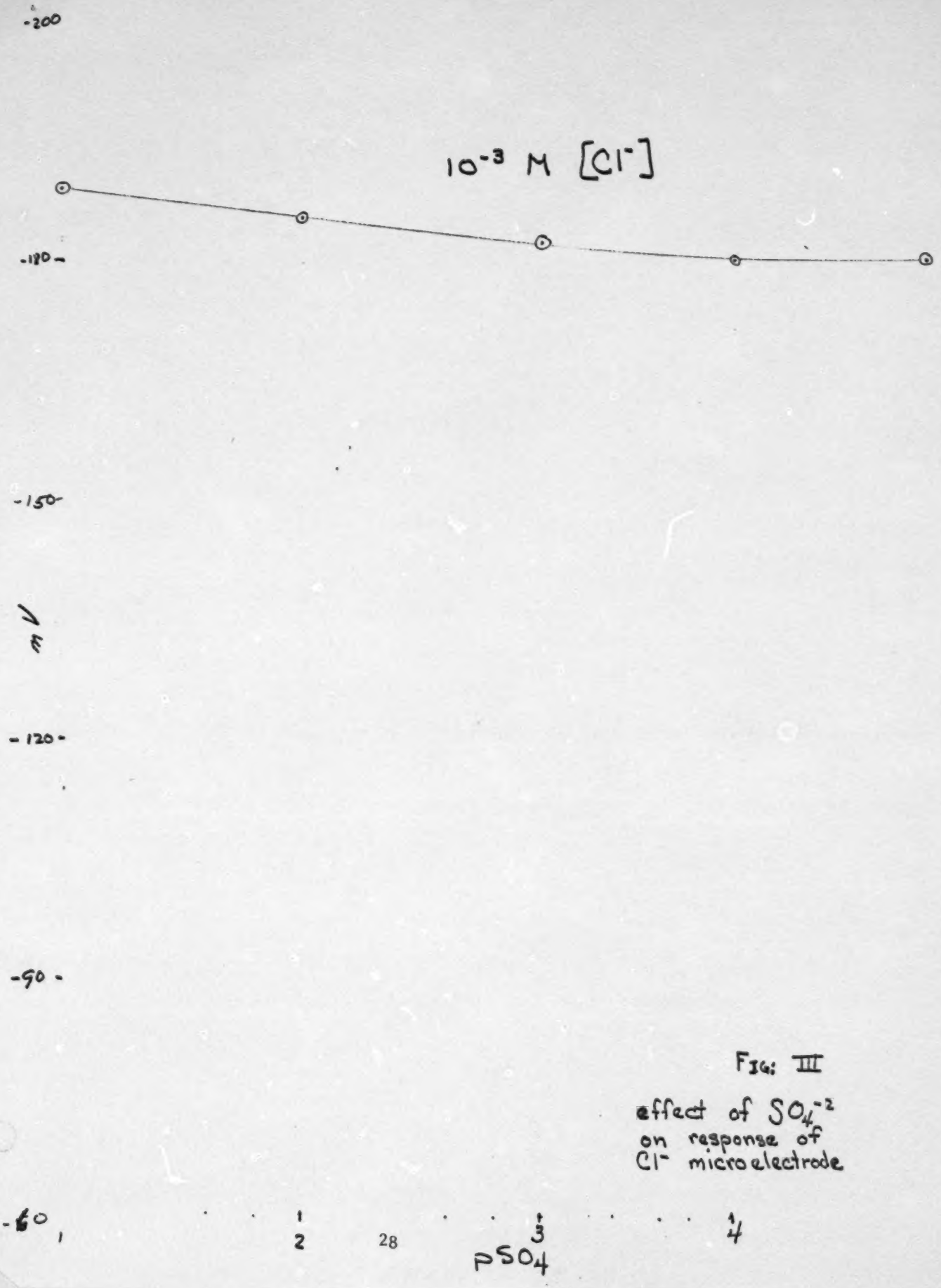
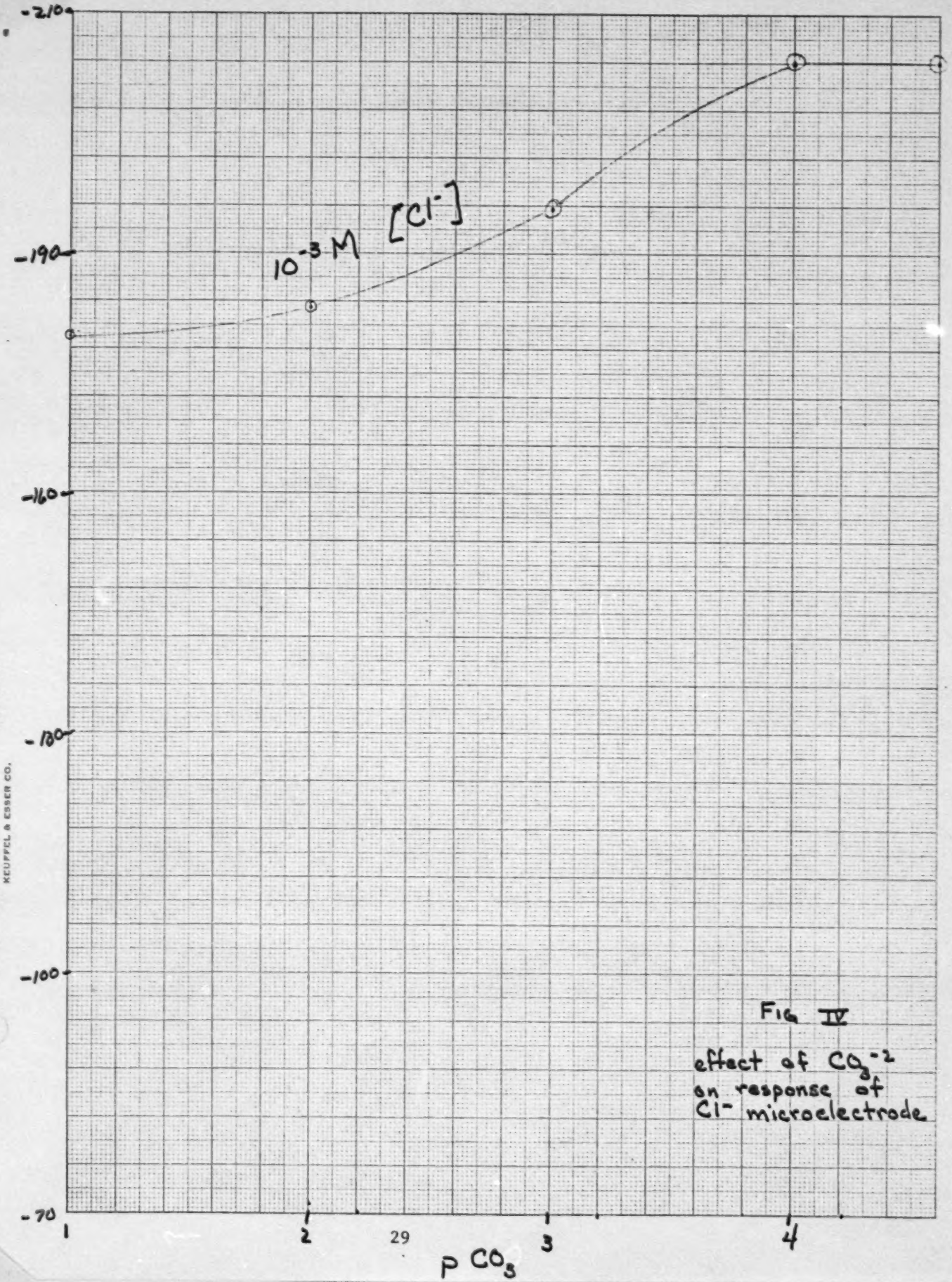


FIG: III

effect of SO_4^{-2} on response of Cl^- microelectrode



K&E 10 X 10 TO THE CENTIMETER 46 1510
18 X 25 CM. MADE IN U.S.A.
KEUFFEL & ESSER CO.

Fig IV

effect of CO_3^{-2} on response of Cl^- microelectrode

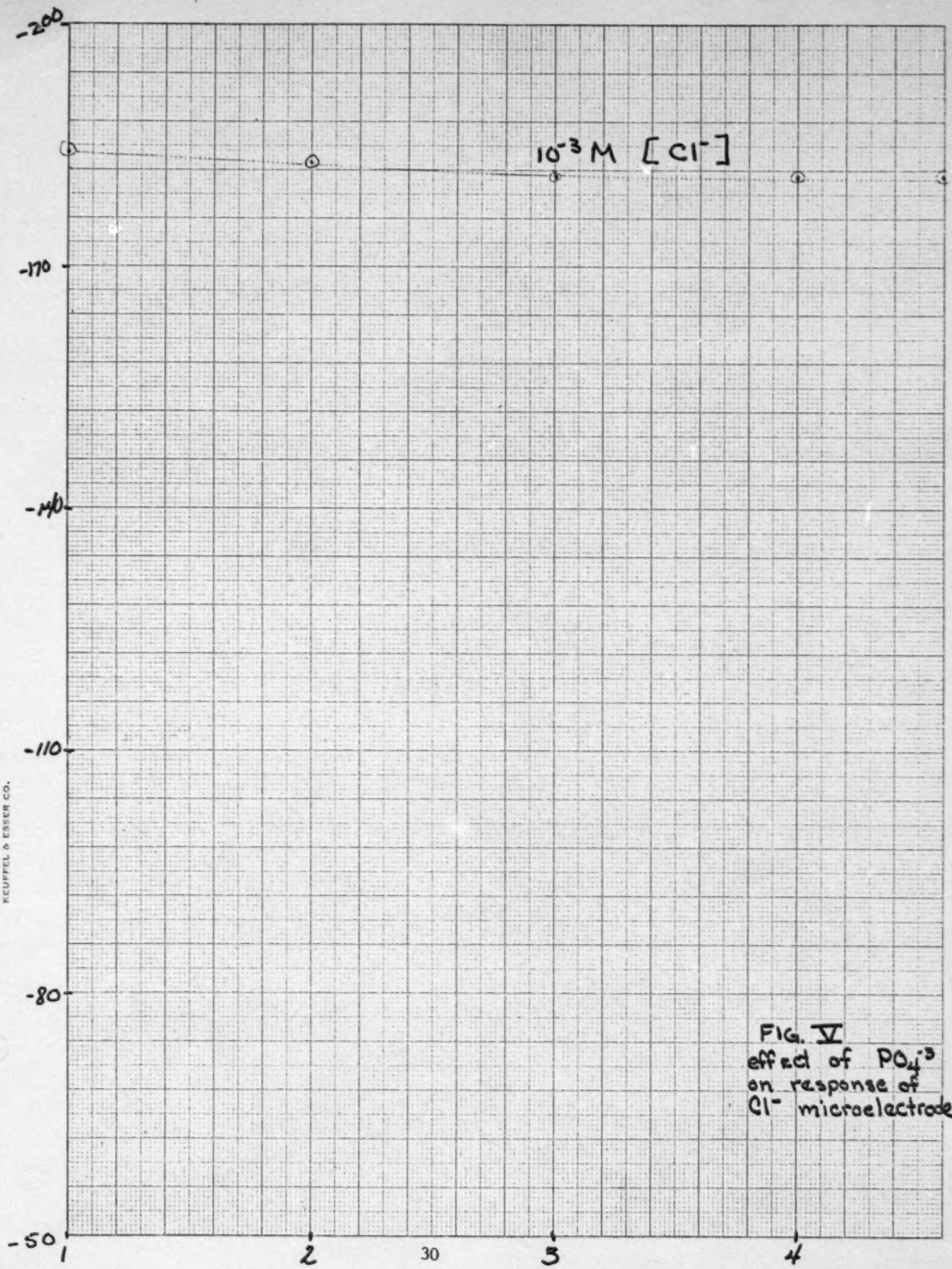
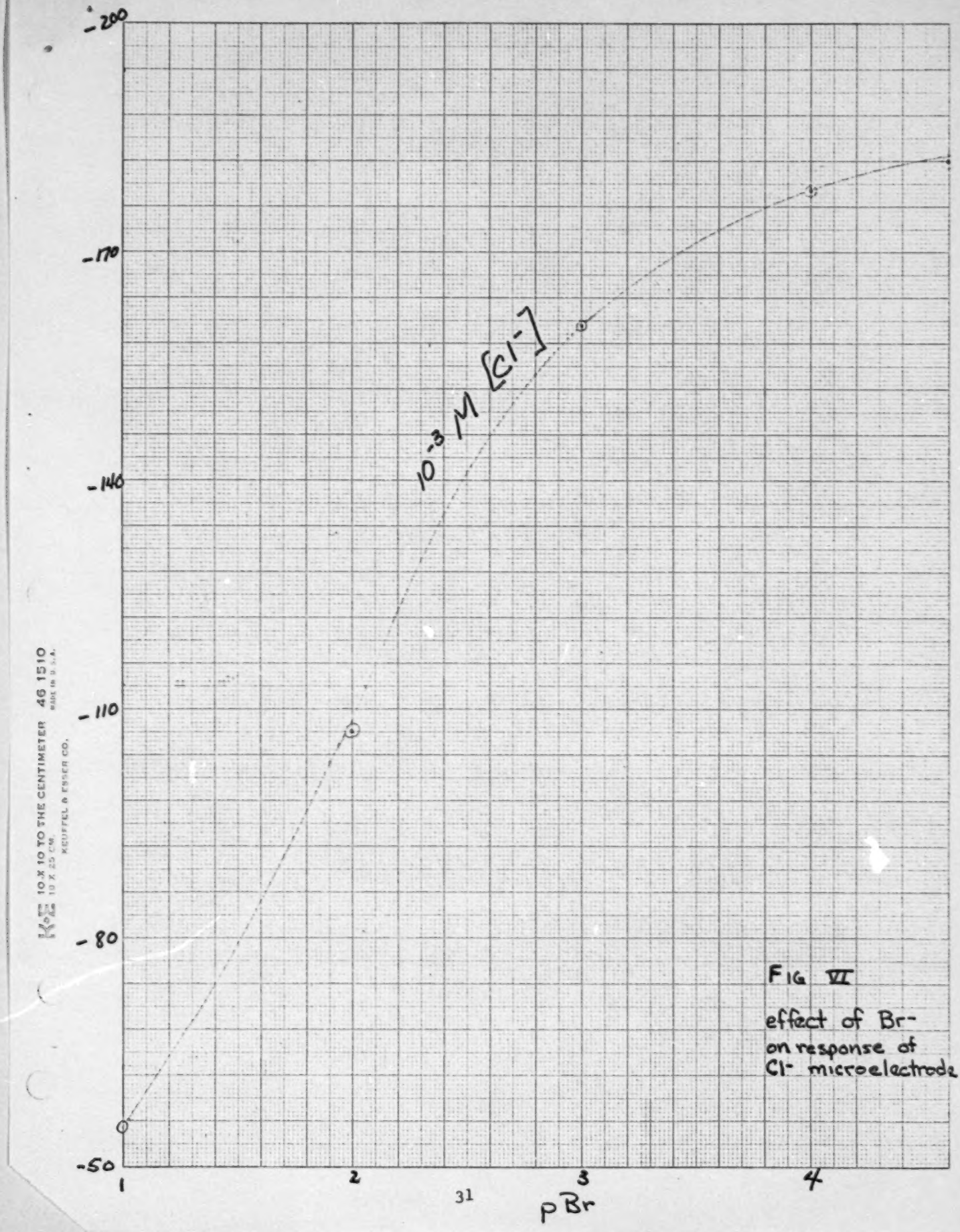


FIG. V
effect of PO_4^{-3} on response of Cl^- microelectrode

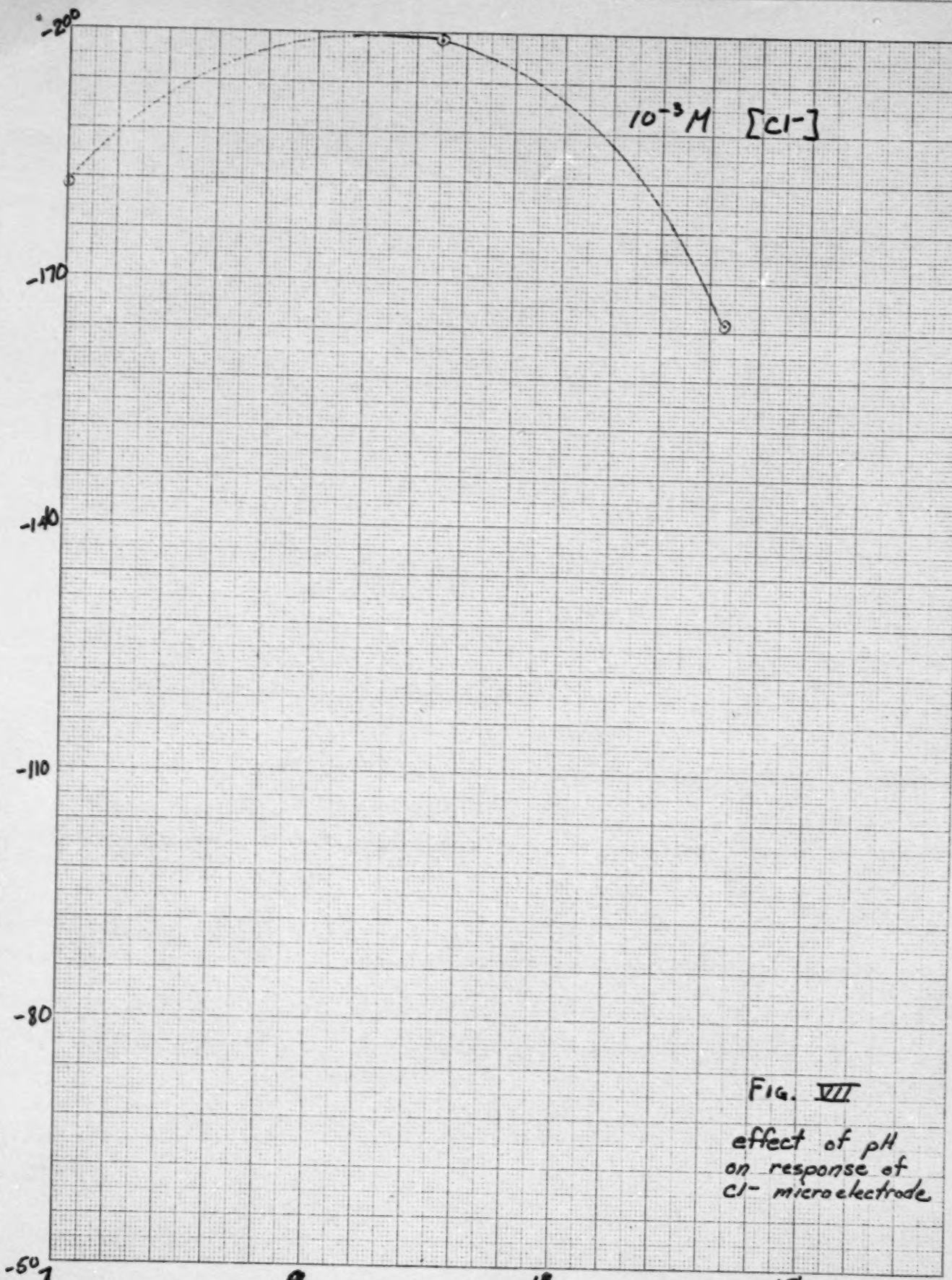
K&E 10 X 10 TO THE CENTIMETER 46 1510
10 X 25 CM. MADE IN U.S.A.
KEUFFEL & ESSER CO.



KEUFFEL & ESSER CO. MADE IN U.S.A. 10 X 10 TO THE CENTIMETER 46 1510 10 X 25 CM.

FIG VI

effect of Br⁻ on response of Cl⁻ microelectrode



KE 10 X 10 TO THE CENTIMETER 46 1510
10 X 25 CM.
KEUFFEL & ESSER CO.
MADE IN U.S.A.

FIG. VII

effect of pH on response of Cl- microelectrode

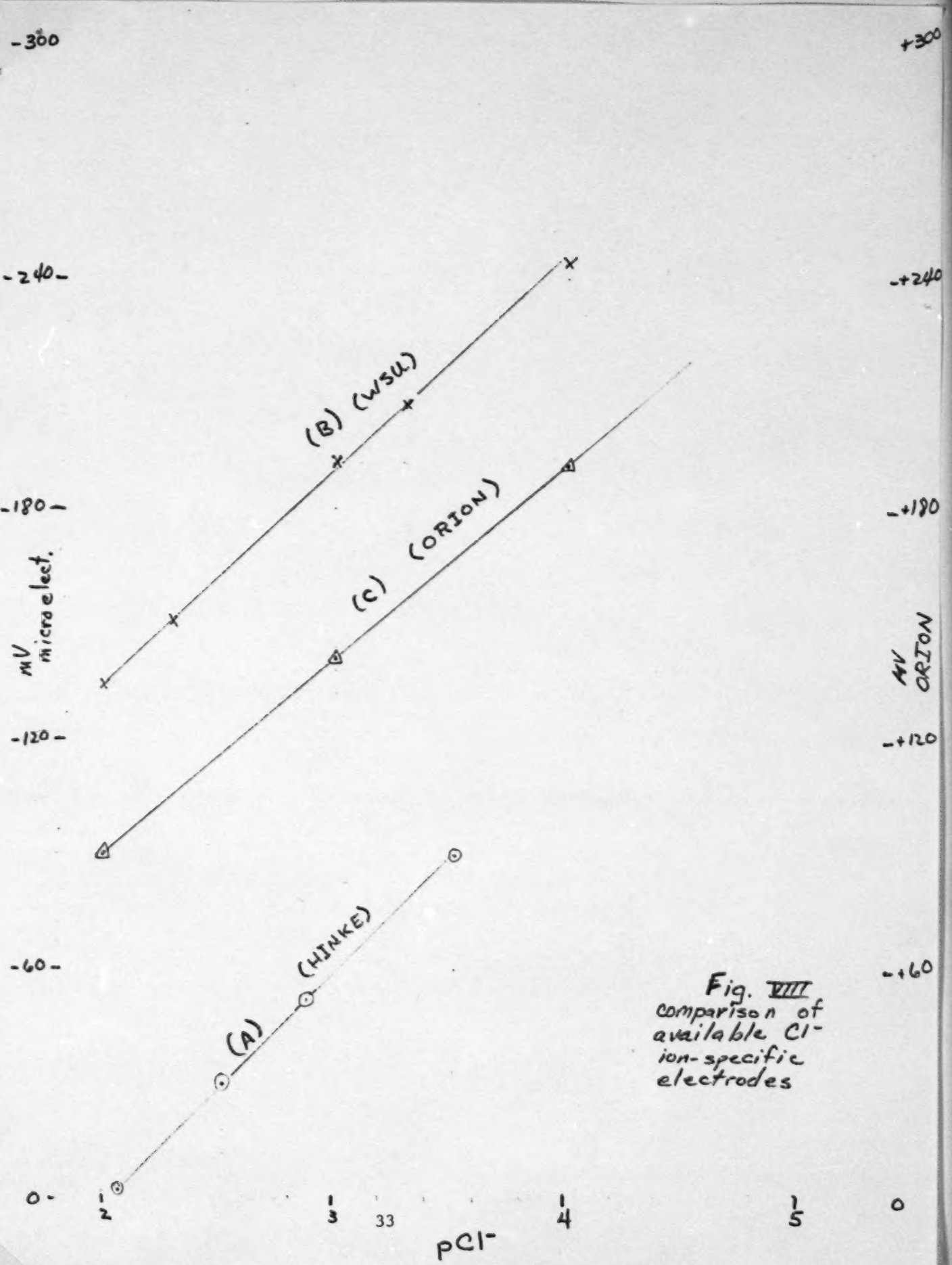


Fig. VIII
comparison of available Cl⁻ ion-specific electrodes

FIGURE IX

BAKELITE MEMBRANE TYPE ELECTRODE

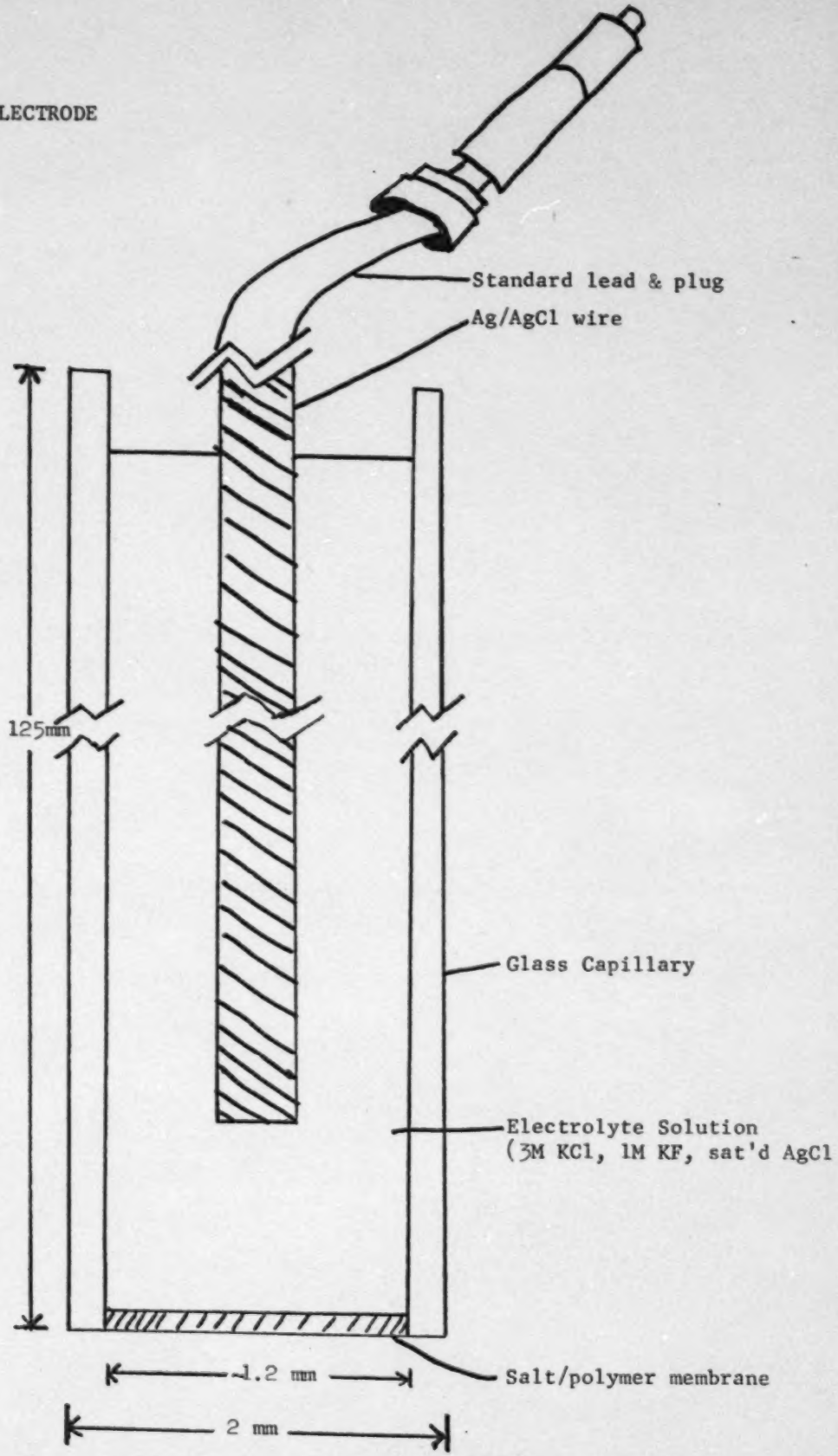
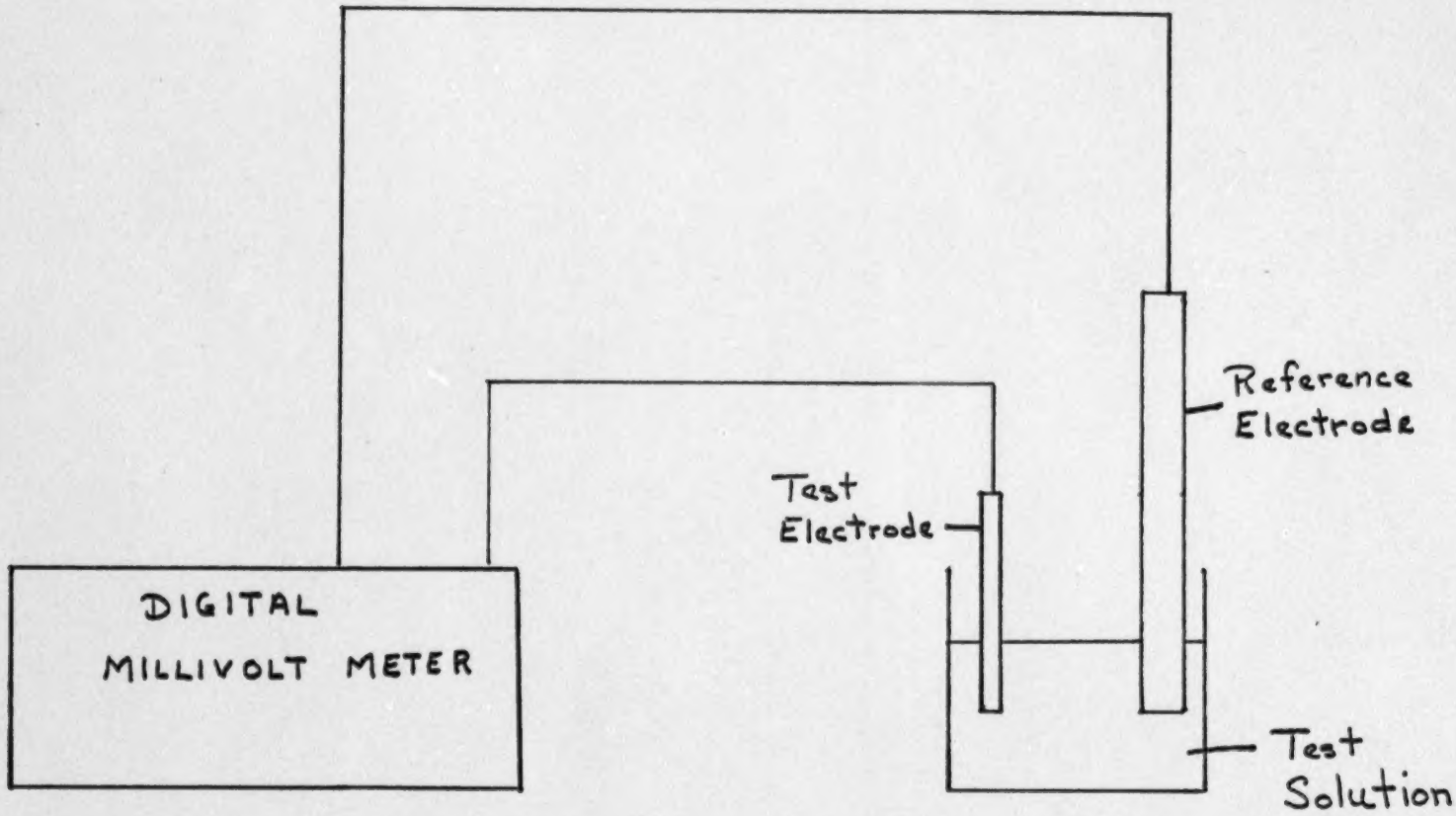


FIGURE X

ELECTRODE TESTING SET-UP



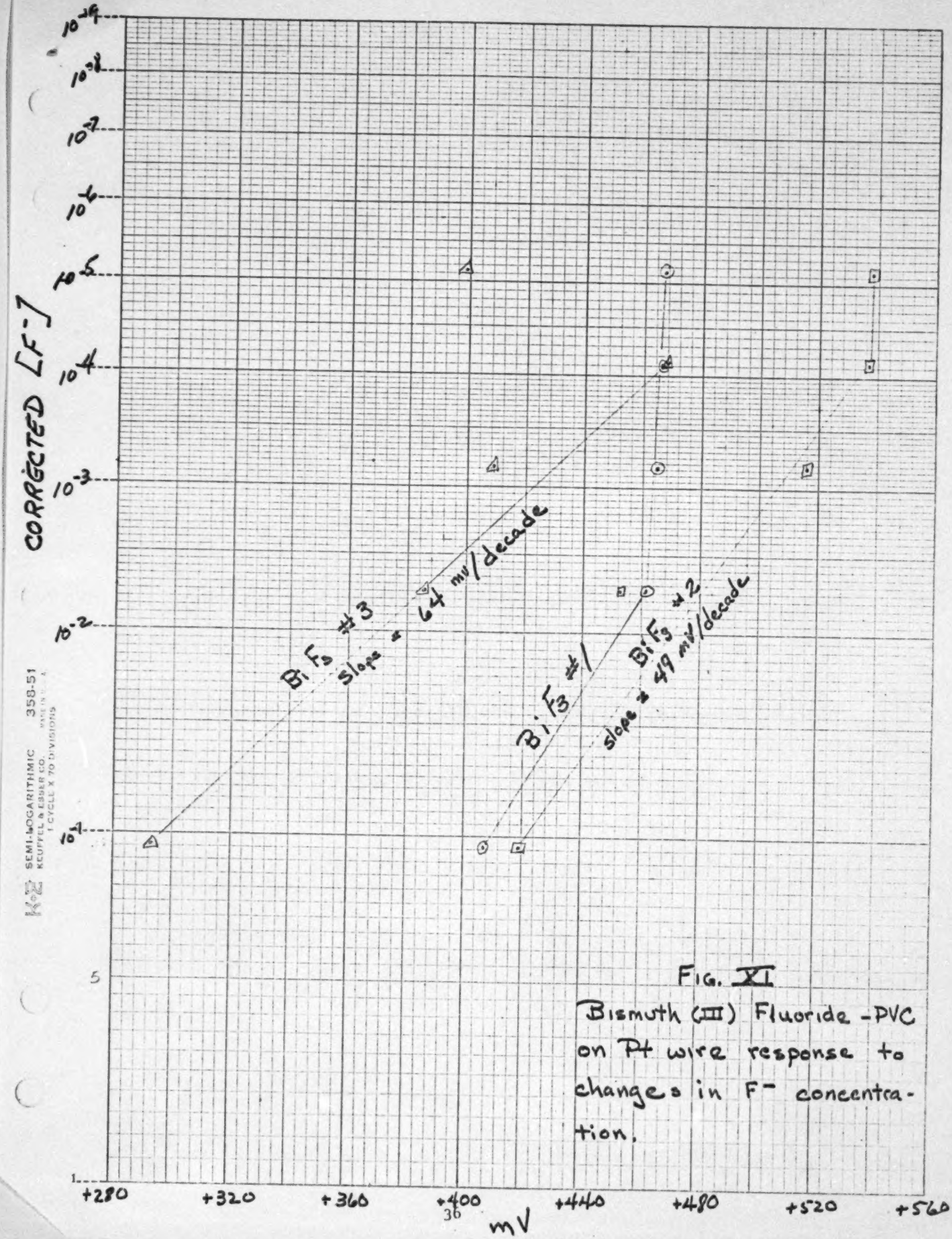


FIG. XI

Bismuth (III) Fluoride - PVC on Pt wire response to changes in F^- concentration.

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